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2,4,5,3',4',5'-HEXABROMOBIPHENYL IS BOTH A 3-METHYLCHOLANTHRENE- AND A PHENOBARBITAL-TYPE INDUCER OF MICROSOMAL DRUG METABOLIZING ENZYMES

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SUMMARY: *The effects of 2,4,5,3',4',5'-hexabromobiphenyl (HBB) on hepatic microsomal drug metabolizing enzymes have been determined. Male rats were injected ip with 90 mg HBB/kg and sacrificed 7 days later. HBB increased liver weight and microsomal protein, and strongly induced NADPH-cytochrome P450 reductase, cytochrome P450, aminopyrine demethylation, benzo[a]pyrene hydroxylation, epoxide hydratase, and p-nitrophenol-UDP-glucuronyltransferase activities, and several microsomal hemoproteins. The microsomal CO difference spectral maximum was shifted from 450 to 448.5 nm. HBB unilaterally causes a mixed-type induction similar to that caused by both phenobarbital and 3-methylcholanthrene and is therefore a highly unusual type of microsomal inducing agent.*

Polybrominated biphenyls (PBBs) have contaminated Michigan's farms and residents, and trace amounts of this chemical have also polluted the environment in New York and New Jersey (1,2). PBBs have been found to result in a mixed-type induction of rat liver microsomal drug metabolizing enzymes (3,4). Such an induction resembles that caused by treatment with the two classical inducers phenobarbital (PB) and 3-methylcholanthrene (MC). While it was expected that the individual PBB congener would be either PB or MC-type inducing agents, it is also possible that one or more congeners, each by itself, could cause both types of responses. We have previously demonstrated that the two major components of PBBs, 2,4,5,2',4',5'-hexa- and 2,3,4,5,2',4',5'-heptabromobiphenyl, which together comprise 83% by weight of the fireMaster® PBB mixture, are strictly PB-type inducers (5,6). However, the MC-type inducer(s) were still unknown. The results described herein demonstrate that one of the remaining PBB congeners has the highly unusual property of being a bifunctional molecule unilaterally capable of causing a mixed-type induction of the microsomal enzymes. This molecule may

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possibly account for the MC-like aspects of the mixed-type induction caused by the fireMaster® PBBs.

EXPERIMENTAL PROCEDURES: Chemicals used in this research were the same as and from the same sources as those used in previous studies on PBBs (5,6). The PBB congener employed in this investigation was purified from fireMaster® BP-6 (Michigan Chemical Corporation). One hundred grams of PBBs were stirred with 200 ml of acetone at room temperature for 2 hours. The acetone-soluble fraction was allowed to stand at 4°C until crystals formed. Successive recrystallization (6 times) in acetone yielded 2,4,5,3',4',5'-hexabromobiphenyl (HBB) at a purity of greater than 99% (yield 250 mg.).

Male Sprague Dawley rats (Spartan Research Animals), weighing between 150 and 180 g, were given a single ip. injection of 90 mg PBBs or HBB per kg body weight in polyethylene glycol (PEG) or 4 ml PEG per kg seven days before sacrifice. PB treated rats were given five daily ip. injections at 50 mg/kg in water and killed on the sixth day. Rats given MC were injected with 20 mg/kg in PEG 36 and 24 hours before sacrifice. Rats subjected to the combined treatment of PB plus MC were given these chemicals at the same doses and schedule used for each alone. Feed (except for the night before sacrifice) and water were available ad libitum. Liver microsomes were isolated, washed, stored and assayed as referenced or described earlier (5,6).

RESULTS: Seven days after dosing with HBB, all of the investigated parameters were substantially altered, (Table I). The liver weight to body weight ratio was increased by 54%, and hepatic microsomal protein was increased to 220% of the control value (per gram liver). The first component of the microsomal mixed-function oxidase system, NADPH-cytochrome P450 reductase, was induced by 40%. PB and PBBs strongly induced this enzyme while MC had no effect. The terminal component, the cytochrome P450 hemoprotein family, was induced to 260 and 160% of control by PB and MC, respectively. The inductions by HBB (to 390% of control) and PBBs were essentially identical to each other but were far greater than those seen in response to either classic inducing agent alone. While PB did not shift λ_{max} in the cytochrome P450 spectral assay from 450 nm, shifts were seen with PBBs (449.5nm), HBB (448.5nm), and MC (448nm).

Aminopyrine demethylation and benzo[a]pyrene hydroxylation were assayed to further determine the PB or MC nature of the induction, respectively (8,9). As shown in Table I, PB, HBB (330% of control), and PBBs all caused comparably strong inductions in aminopyrine demethylation, while MC had little effect on this activity. HBB was also an excellent inducer of benzo[a]pyrene

TABLE I. EFFECT OF VARIOUS PRETREATMENTS ON SEVERAL LIVER MICROSOMAL DRUG METABOLISM PARAMETER[1].

	PARAMETERS							
TREATMENT	LIVER WT. percent of body wt.	MICROSOMAL PROTEIN mg/g liver	CYT.P450 nmoles/mg	CYT.P450-REDUCTASE nmoles/min·mg	UDPGT nmoles/min.mg	AMINOPYRINE DEMETHYLASE nmoles/min·mg	BENZOPYRENE HYDROXYLASE nmoles/min·mg	EPOXIDE HYDRATASE nmoles/min.mg
None	4.4 ±0.3	7.48 ±0.84	1.00 ±0.07	278.5 ±31.6	33.2 ±11.8	5.1 ±1.9	1.85 ±0.45	5.42 ±1.17
HBB	6.8 ±0.6	17.45 ±2.88	3.88 ±0.28	393.0 ±24.8	81.0 ±10.6	16.8 ±0.5	15.59 ±2.95	16.00 ±1.85
PB	5.6+ ±0.6	13.67 ±1.96	2.63 ±0.25	479.0 ±39.1	30.3* ±1.3	17.8 ±0.6	3.74 ±0.61	12.23 ±1.70
MC	5.1 ±0.2	10.48+ ±1.41	1.63 ±0.10	277.0* ±11.1	68.0 ±12.3	7.2* ±1.3	21.30 ±1.29	5.99* ±1.12
PBB	6.9 ±0.3	21.73 ±1.01	3.65 ±0.25	543.8 ±26.0	60.4 ±6.1	17.2 ±1.4	10.04 ±0.74	18.73 ±1.53

1. Mean ± standard deviations for four animals. All values are statistically different from controls ($p<.001$), except + where $p<.05$, and * where values are not statistically different from controls.

hydroxylation (840% of control), though it was not quite as effective as MC alone. PBBs (and PB plus MC, data not shown) also caused major inductions to half the maximal level, while PB only doubled the control activity.

Epoxide hydratase is inducible by PB, not MC (10), while p-nitrophenol-UDP-glucuronyltransferase (UDPGT) is inducible by MC but not PB (11). PBBs are known to strongly induce both (5,6). As shown in Table I, HBB was capable of causing major inductions of both enzymes, while the well established effects of the other agents were verified.

The effect of HBB on microsomal protein and hemoprotein profiles was examined by SDS-polyacrylamide gel electrophoresis. This technique has been successfully employed to differentiate between the PB- and MC-type cytochrome P450 hemoproteins (12,13). Figure 1 shows the effects of different agents on the profiles of microsomal proteins (top) and hemoproteins (bottom). The pattern of induction following treatment with HBB shows that at least two distinctly different hemoproteins were markedly induced by treatment with both PB and MC. As has been previously demonstrated, PBBs also induce at least two hemoproteins, (5,6), but HBB appears to cause more induction of the MC-inducible hemoprotein(s) than does the PBB mixture. A gel of microsomes from an animal pretreated with 2,3,4,5,2',3',4',5'-octabromo-biphenyl, strictly a PB-type inducer (14), is included for comparison.

In addition to these biochemical effects, HBB had significant effects on several physiological parameters. Body weight gain over the seven day period was only 1.1 g/day, while PBB-treated rats gained 2.2 g/day and control rats gained an average of 3.2 g/day. Thymus gland weights were less than 50% of control, and pathological changes were noted in several tissues. All of these parameters are the subject of further investigation.

DISCUSSION: HBB clearly caused a mixed-type induction of hepatic microsomal drug metabolizing enzymes. The major increases in NADPH-cytochrome P450 reductase, aminopyrine demethylation, and epoxide hydratase activities demonstrate its PB-like effects, while the inductions in benzo[a]pyrene

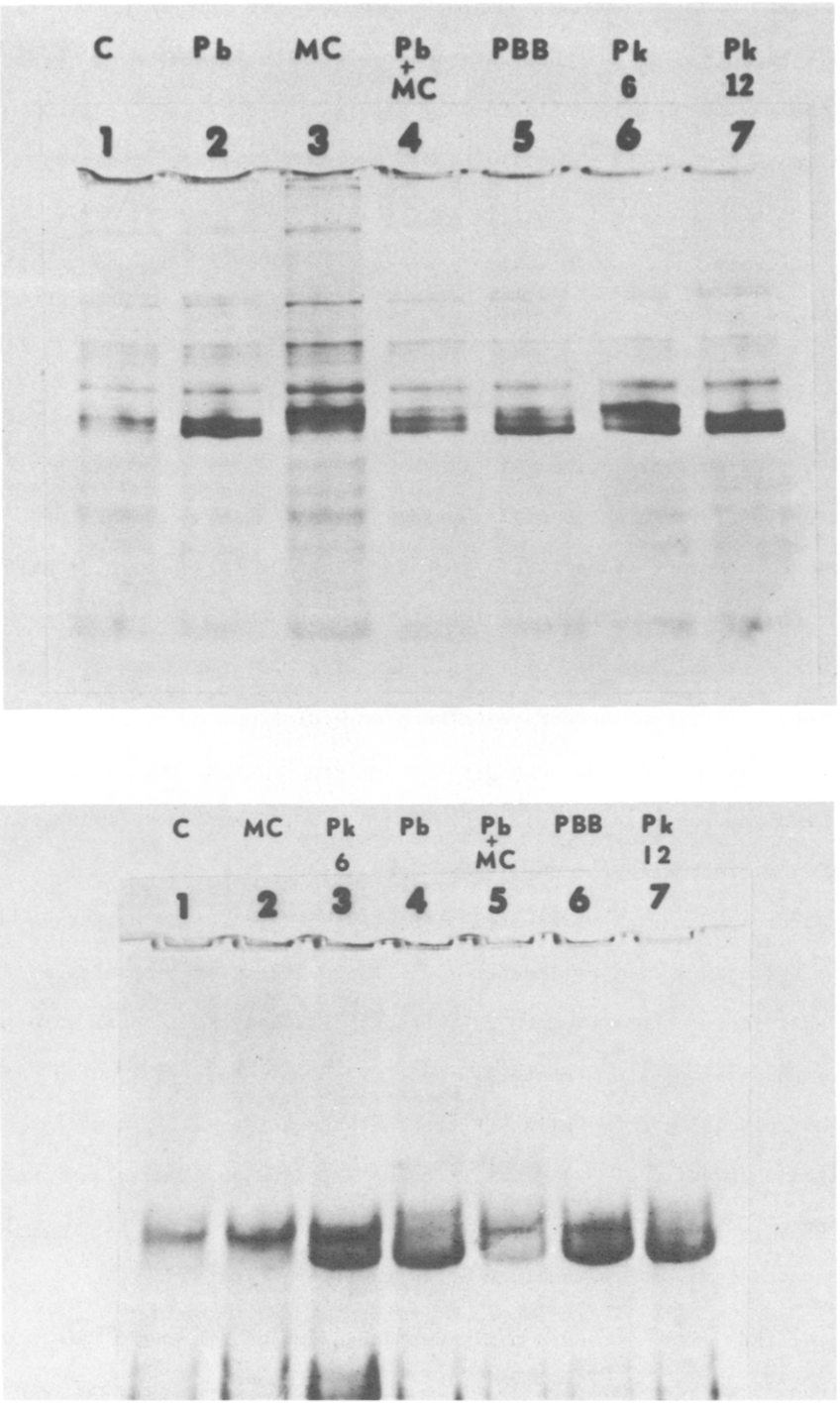

Figure 1. Effects of HBB and PBBs on the protein (top) and heme (bottom) profiles of microsomes subjected to SDS-polyacrylamide gel electrophoresis. Heme staining was performed on gels containing 120 μg of microsomal protein,

hydroxylation and UDPGT activities demonstrate its MC-like effects. The cytochrome P450 spectral maximum and the results of SDS-polyacrylamide gel electrophoresis each also demonstrate that a mixed-type induction has occurred.

If HBB is by itself capable of causing a mixed-type induction, then it may (with one possible exception) represent the first example of a unique class of inducing agents. 2,4,5,2',4',5'-(15), 2,3,4,2',3',4'-(16), and 2,3,4,2',4',5'-Hexachlorobiphenyl (16) have each been reported to be mixed-type microsomal inducers, but Goldstein et al. (17) have now shown that a contaminant was responsible for the MC-like aspects of the induction caused by a 2,4,5,2',4',5' isomer, and that chlorinated dibenzofurans contaminants most likely also explain the MC-like aspects of the induction seen with the other two chlorobiphenyls. It is known that these contaminants are formed during the synthetic process used to prepare the other two hexachlorobiphenyls (18,19). Hexachlorobenzene has also been proposed as a mixed-type inducer (15,20), but if it indeed has both PB- and MC-like effects, then they are quite weak, since hexachlorobenzene is a poor inducing agent. Although not known to contain potent contaminants, the purity of the hexachlorobenzene used in these studies was not well established.

The question of the purity of HBB is the central issue. The HBB used in the experiments reported here was determined to be 99.62% pure by electron capture has chromotography. The contaminants had retention times identical to those of 2,4,5,2',5'-penta- (0.04%), 2,4,5,3',4'-penta- (0.24%), and 2,4,5,2',4',5'-hexabromobiphenyl (0.10%) (7). In order to rule out the possibility that the mixed-type induction was not due to contaminants such as brominated dibenzo-p-dioxins or dibenzofurans, additional purification procedures were employed, and the experiment repeated. The HBB was dissolved in hexane and passed over activated charcoal (Mallinckrodt). Next it was

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while 40 μg of microsomal protein was applied to gels stained for protein (13). Microsomes are from control rats (C) or from rats pretreated with 3-methylcholanthrene (MC), phenobarbital (Pb), 3-methylcholanthrene plus phenobarbital (Pb + MC), fireMaster (PBB), 2,4,5,3',4',5'-hexabromobiphenyl (Pk 6), or 2,3,4,5,2',3',4',5'-octabromobiphenyl (Pk 12).

passed over a neutral alumina column with hexane (7). Gas chromatographic analysis under essentially the same conditions as used by O'Keefe (23) revealed no brominated dibenzofurans or naphthalenes. No other compounds could be detected by gas chromatographic analysis from 190° to 320°. No other substances could be detected by GC-MS (LKB 9000), nor by temperature programming a mass spectrometer direct probe (Varian CH5) from 20° to 180°. The latter would have been capable of detecting a 0.01% contaminant. When the highly purified HBB was used in a duplicate experiment, the results were essentially identical to those obtained in the first experiment.

It is possible that one or more metabolites of HBB could be a different type inducing agent than the parent molecule and thereby account for the mixed-type induction. However, HBB does not appear to be metabolized *in vitro* (24

Several researchers have investigated the effects of chlorinated biphenyls on microsomal drug metabolizing enzymes. *Meta* and *para* chlorination was found to be essential for an MC-type induction, but *ortho* chlorination prevented this response, presumably by disrupting planarity (25,26). Chlorination at both the *ortho* and *para* positions on both rings was found to provide the best PB-like response (26). Kohli *et al*. have recently found an exception to these conclusions, when they determined that 2,3,5,2',3',5'-hexachlorobiphenyl was a good PB-type inducer (27). HBB represents another exception, in that an MC-type response was seen despite the presence of one *ortho* bromine. The degree of planarity of HBB is uncertain, although its ^{1}H-NMR spectrum demonstrates that the rings can rotate rapidly at room temperature in organic solvents (6). It had previously been believed that the mixed-type induction seen in response to halogenated biphenyl mixtures was due strictly to the combined effects of molecules that by themselves were either PB- or MC-type inducers but not both. HBB is the first halogenated biphenyl found to be a mixed-type inducer.

Brominated naphthalenes have been found to constitute approximately 220 ppm of the fireMaster® PBB mixture (28), but they are not potent enough

MC-type inducers to account for this aspect of the mixed-type induction caused by fireMaster® (29). Brominated dibenzo-p-dioxins and dibenzofurans have not been found in fireMaster® (<0.5 ppm); if present, they or other polar contaminants do not appear to be responsible for the toxic effects of PBBs (28). While 3,4,5,3',4',5'-hexabromobiphenyl is a potent MC-type inducer (25), it too appears to be absent from fireMaster® (30). 2,4,5, 2',4',5'-Hexa-(5), 2,3,4,5,2',4',5'-hepta-(6), 2,3,4,5,2',3',4',5'-octabromobiphenyl (14), and HBB each contribute to the PB-like aspects of the induction caused by the PBB mixture. The extent to which HBB contributes to the MC-like aspects of the PBB induction is now being investigated.

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